

# Animal Chlorophyll: its Relation to Haemoglobin and to other Animal Pigments.

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PART I. THE PIGMENTS OF ANIMALS HAVING  
NO BLOOD-VASCULAR SYSTEM.

1. INTRODUCTION.

IN the study of marine invertebrates one of the most impressive things encountered is the great richness and variety of colour; it is not surprising, therefore, that the question of animal coloration has long engaged great attention. Investigated at first superficially by those who sought an explanation of the so-called phenomenon of 'protective coloration', the problem attracted, during the latter part of the nineteenth century, the attention of several English physiologists, and it is to the investigators of this group—Lankester, Sorby, MacMunn, Mosley, Griffiths, Poulton, and Halliburton are the more important names—that we are indebted for very real contributions to our knowledge of animal pigments; especially for the introduction of the microspectroscope into this field of biological research. Since 1900 the work on pigmentation has been to a very large extent spasmodic, and while certain valuable additions have been made (in particular, those of Gamble and Keeble) there are still many problems which invite further investigation.

The present paper aims to show that the pigment which is responsible for the colour of certain representative invertebrates comes from the blood-stream, and that in many cases the pigment cells of the blood arise (while in circulation) from unpigmented corpuscles. This view concerning the origin of pigment occurred to the writer after noting that a pigment of the blood appeared to be identical with the body pigment in three representative phyla: (1) in another paper the writer (Fulton, 1921 *b*) has shown that the pigmented corpuscles in the blood of a Bermuda tunicate, *Ascidia atra*, arise in the blood from colourless cells, and that the blue pigment cell, so common in the blood-stream, is identical with the blue cells of the tunic—the cells which give to this ascidian its intense blue colour; (2) Crozier (1916 *b*) has demonstrated that the blue

pigment granules which colour the nudibranch *Chromodoris zebra* are also to be found in great abundance in the pigment cells of the blood—the identity of the two pigments having been determined by the spectroscope; (3) lastly, while examining the body-fluid of one of the common sea-urchins, *Tripneustes esculentus*, it was found that the large red amoeboid cells (so well described by Geddes, 1880) gave strong indication that their pigment is the same as that which colours their spines and tube-feet. Thus, in a tunicate, a mollusc, and an echinoderm there seemed to be very good evidence that the body-pigment is found also in the blood.

The experimental work was carried on at the Bermuda Biological Station for Research during the summer of 1920, and I wish to express my warmest thanks to Dr. E. L. Mark, who gave me the facilities of the laboratory, and who made possible my trip to Bermuda.

## 2. PROTOZOA AND PORIFERA.

With any effort to trace animal pigment back to the blood-system there arises at once a very serious difficulty. Pigmentation as such appears phylogenetically long before the rise of the blood-system. How, then, is it possible to assume that all coloration comes from the blood?

Among the Protozoa the only class—with rare exceptions—which contains chromatophores is the Mastigophora (Minchin, 1912, p. 13). Here, however, the pigment is probably in every case chlorophyll, or a substance closely allied to chlorophyll. A typical species of chlorophyllogenous protozoa is *Archeria boltoni*, which was described by Lankester (1885). One notable exception to the assumption that all protozoan pigments are closely related to chlorophyll is found in *Stentor cæruleus*, which possesses a blue pigment called by Lankester (1873) 'stentorin'. Spectroscopically the absorption bands of this pigment, quite unlike chlorophyll, resemble those of the blue algal pigment, phycocyanin, which, according to Phillips (1911, p. 596), when present even in minute amounts, greatly alters the spectrum of chlorophyll. Consequently

stentorin is not to be considered an important exception to the rule that all protozoan pigments are chlorophyllogenous in nature.

But how does the chlorophyll of protozoans originate? Is the animal itself capable of manufacturing chlorophyll, or is it the result of outside infection? Geddes (1882) and Lankester (1882 *a*, and 1882 *b*) maintained strenuously that *Hydra viridis* and *Spongilla fluviatilis* were capable of synthesizing their own chlorophyll:<sup>1</sup> that the green deposits found in these animals are chloroplasts belonging to the animal and consequently are not of plant origin. In support of his contention Lankester asserted the absence of nucleus and cellulose wall in the green corpuscles. Though no histological evidence has been adduced to show the presence of a nucleus in these bodies, it seems fair to conclude, since Beyerinck (1890, note 1, p. 784) has succeeded in obtaining cultures of algae from the green corpuscles of *Hydra viridis*,<sup>2</sup> that the chlorophyll of *Hydra* is algal in nature and due to an infection from the outside. The algae probably represent a phase in the life-history of *Chlorella viridis*. A similar condition undoubtedly holds for most of the green protozoa: Famintzin (1889, 1891), Dantec (1892), and Dangeard (1900) all report having obtained colonies of algae from the macerated tissues of *Stentor*, *Paramoecium*, and *Frontonia*; Schewiakoff (1891) found that if colourless *Frontonia* are fed upon macerated green specimens they become infected with green algae which subsequently divide within the cell. Similar results have been reported for *Paramoecium* (Dantec). Certain contrary evidence is also on record for the Protozoa—the puzzling cases of *Vorticella campanula* (Englemann, 1883), and of *Pelomyxa viridis* (Bourne, 1891)

<sup>1</sup> For a more complete discussion of this question see Gamble and Keeble (1903), Keeble and Gamble (1907), and Fulton (1921 *a*).

<sup>2</sup> Entz (1881 and 1883) reports similar results, but in his experiments little precaution was taken against infection and consequently the results are to be accepted with caution. Recently Goetch (1921) has succeeded in infecting colorless *Hydra* with *Chlorella*, and in so doing has corroborated in a very substantial way the views of the earlier investigators cited above.

are the most significant examples—which favours Lankester's hypothesis of the intrinsic nature of certain of the animal chlorophylls. Nevertheless the balance of evidence seems to favour the algal theory to account for chlorophyll in animals; and it is probable, moreover, that further investigation with a more refined technique will show conclusively that Lankester was wrong. Particularly does this seem probable in the light of the classic researches of Gamble and Keeble (which will be discussed later) on *Convoluta*, the green cells of which were shown to be intruding algae. In short, the Protozoa do not present any real difficulty to assuming that body-pigments arise in the blood.

The sponges possess nothing in the nature of a blood-system: nutrition and respiration being accomplished by water currents within the body. What, then, is the nature of their coloration? Is it an animal pigment, which has arisen independently of the blood-system; or is it, as in the Protozoa, a chlorophyllous substance? Though it has not been demonstrated that every species of sponge contains chlorophyll, the spectroscopic investigations of Sorby (1875 *a*), Krukenberg (1884), and MacMunn (1888) have established the presence of a chlorophyllous pigment in eighteen species of sponge, it being most common in the genera *Halichondria* and *Halina*. The more highly-coloured sponges possess pigments the absorption bands of which (as indicated by the figures of MacMunn and Krukenberg) resemble in many respects those of certain of the pigments from blue and red algae, the pigment spectra of these sponges is, in addition, quite different from the spectra of chlorophyll. Inasmuch as it has been shown that the presence of small quantities of such algal pigments as phycoeyanin, phycophaein, and phycoerythrin (Phillips, 1911; also Willstätter and Stoll, 1913) greatly alter the spectrum of chlorophyll, the fact that the pigment of certain coloured sponges fails to show the bands characteristic of the plant pigment does not necessarily indicate its absence. As in the Protozoa, one is also confronted in the Porifera with a question concerning the nature of the pigment itself—a point which is as yet unsettled. Lankester (1882) and his school were vigorous in upholding the animal origin

of sponge chlorophyll, while Brandt (1881 *a*, 1881 *b*, 1882, and 1883) supported the view that chlorophyll in the Porifera and other animals results from a symbiotic association with green algae.<sup>1</sup> Zooxanthellae—symbiotic holophytic flagellates—have been reported for several sponges, also recently by Kirkpatrick (1912) for *Merlia normani*.<sup>2</sup> Cotte (1904) likewise gives an account of an interesting association of this sort.

There is no doubt but that Lankester had every reason to question the evidence of Brandt—which in the light of later investigations was most decidedly inconclusive—and he 'has done valuable service by his championship of the opposed view, that of the intrinsic nature of the corpuscles under discussion. For his view compels those who hold the "algal" theory to investigate each case separately and to vindicate their view by the synthesis of the green animal' (Keeble and Gamble, 1907, p. 171). Now, however, there is little question but that true chlorophyll in animals owes its existence in every case to plants. It seems evident, therefore, that the pigmentation of most sponges has resulted from an association, symbiotic or otherwise, with plant cells; and that, as with the Protozoa, the Porifera present no serious obstacle to the assumption that animal colour arises in the blood. Consequently a discussion of the phylogenetic aspect of pigmentation must of necessity commence with the coelenterates.

### 3. COELENTERATA.

Since the coelenterates are organisms having but two cell layers, ectoderm and entoderm, it is at once obvious that they

<sup>1</sup> Since the present writing the work of Van Trigt (1918) has been brought to the writer's attention. He has shown that in *Spongilla* the green cells very clearly are invading organisms, and has made an extensive series of experiments with cultures of the green cells derived from the macerated sponge tissue. He has also given conclusive proof of an oxygen-carbon dioxide exchange between the algal cells and the sponge tissue. His evidence further corroborates the view just expressed concerning the symbiotic nature of the green cells in sponges.

<sup>2</sup> Winter (1907) has shown that Zooxanthellae are symbiotic in the foraminifer *Peneroplis*.

can possess no blood-system in the sense in which it is used for the higher animals. Nevertheless, as Griffiths (1892, pp. 128 and 184) has emphasized, the nutritive or 'chylaqueous' fluid is analogous to the blood of the higher forms in that it carries nourishment, supplies oxygen, carries off the waste products of metabolism, and in many cases, as Kollmann (1908) and others have since shown, is a corpusculate fluid. The importance of this analogy between the chylaqueous fluid and the blood of higher animals is uncertain; however, a question immediately presents itself concerning the relation of this fluid to the pigmentation of the coelenterates. It is therefore desirable to consider first the nature of coelenterate pigments. The animals on which the greater share of my work has been done are two species of actinians common in the Bermuda Islands: *Condylactis passiflora* Duch. and Mich.,<sup>1</sup> and *Actinia bermudensis* Verill.

(a) *Condylactis passiflora*.

*Condylactis* occurs in great abundance in all parts of the Bermudas, and is usually found firmly attached to the under side of rocks and in crevices just below the level of low tide.

If the gastrovascular (chylaqueous) fluid of *Condylactis* be withdrawn at any point on the body with a hypodermic syringe and examined, two types of cell are usually to be observed: one a yellow cell with several large granules, and the other, an unpigmented element. The pigment cells of this body-fluid might easily be confused with wandering pigment cells of the body-wall. In reality, however (as Rand, 1909, has noted) these cells are Zooxanthellae, and it is this fact which in part explains a very striking phenomenon presented by a fresh smear of the gastrovascular fluid: viz. the very marked oscillation of the individual cells. These yellow cells (Zooxanthellae) gyrate usually in a counter-clockwise direction on a single axis, while the colourless cells as a rule

<sup>1</sup> For an excellent description of this species McMurrich's (1889) paper should be consulted.

vibrate much less regularly. The latter, inasmuch as they are motile, have in all probability been torn from the ciliated lining of the gastrovascular cavity; with acetic acid or neutral red their cilia may very easily be demonstrated.

The coloration of *Condylactis*, it should be emphasized, is due largely, but not entirely, to this yellow flagellate. The tissue of the tentacles is itself colourless (Rand, 1909; Parker, 1917), as is shown when a tentacle is transected. The brownish-yellow colour, which is a constant feature of the uninjured tentacle, is therefore due to the presence of the flagellate organism in the internal fluid. This may readily be shown by examining the liquid contents of the tentacle. When an animal is withdrawn from the aquarium with the tentacles in expanded condition the internal pressure on the gastrovascular fluid causes minute streams of water to issue from the terminal pore of each tentacle. If some of the fluid so exuded be caught in a watch-glass it occasionally contains the 'symbiotic' organisms; under normal conditions of exudation, however, they probably do not escape when the tentacle contracts.

Not only are the tentacles coloured by the presence of *Zooxanthellae*, but the column itself owes much of its colour to this organism. However, the column also possesses large collections of red pigment granules, some patches being as much as 2 mm. in diameter. These are more highly concentrated in the lower parts of the column than in the upper, which gives to the basal region an intense red colour, while the upper parts tend toward the brownish yellow of the tentacles.

In two *Siphonophora*, *Velella spirans* and *Porpita umbella*, Kuskop (1921) has found *Zooxanthellae* in abundance; they reside chiefly in the 'hepatic canals', so called, and in the gonophores. Their occurrence in the latter organs strongly suggests that the association of the *Zooxanthellae* with these coelenterates is continuous from one generation to another. Before discussing the significance of these observations the condition of the pigment in *Actinia bermudensis* will also be described.



*(b) Actinia bermudensis.*

*Actinia bermudensis* is a deep red anemone, which is found on the rocks just about the level of low tide. At high tide, when the water splashes over them, their tentacles open up for feeding; when out of contact with the water they draw their tentacles into the interior of the column and have the appearance of a deep-red gelatinous mass hanging limply from the rocks. The specimens used in the present study were obtained from the caves on the north side of Long Island (Bermuda) where they occur in considerable numbers. This species is distinguished by a very remarkable power of resisting unfavourable surroundings; as an example, it will remain alive sealed in a 100 cc. of sea-water for from six to seven days (Fulton, 1921 *a*).

*A. bermudensis* is coloured uniformly by red pigment granules, which are spread through the entire ectoderm. The granules are not of a definite size, however, and the outlines of the cells which contain them are never clear in the living tissue and can be discerned only with great difficulty in tissue which has been fixed. In shade, the pigment is precisely the same as the red pigment patches of *Condylactis*. Consequently a series of experiments was performed with a view to determining whether or not the two pigments are identical. Small pieces of tissue from each species were teased out and placed side by side upon a slide. Their action in the presence of an acid was first tested. In both cases when either 10% hydrochloric acid or 10% valeric acid<sup>1</sup> were added a decided increase in the depth of colour took place. When treated with alkalis ( $\text{NH}_4\text{OH}$  and  $\text{NaOH}$ , 10% and 50%) no change could be observed. Neither of the pigments could be dissolved with any of the following solvents: ether, chloroform, methyl, ethyl or amyl alcohol, petroleum ether, xylol, or pyridine. In acetone, however, the pigment of *A. bermudensis*

<sup>1</sup> It has been shown by Crozier (1915, 1916 *a*, and 1916 *c*) that, of twenty-two of the more common acids, valeric is the most penetrating to tissues.

proved readily soluble; the condylactid pigment was also dissolved by acetone, but not so readily. The difference in rate of dissolution is probably to be accounted for by the greater thickness of the *Condylactis* tissue. Therefore, since the two pigments are found in species of the same order, since they are of the same colour, since in the presence of acid they are uniformly deepened in shade, and since they are each to be extracted by only one (the same one) of nine solvents, it seems reasonable to conclude that the two pigments are identical.

Among the earlier authors the observations of Moseley (1873) upon actinian pigments are perhaps the most significant. He described a red colouring matter (from *Actinia mesembryanthemum* and from *Bunodes crassicornis*) which he called 'actiniochrome'; MacMunn found this pigment insoluble in many of the ordinary solvents for animal pigments.<sup>1</sup> All of the solvents which he employed also gave negative results when applied to *A. bermudensis* and *C. passiflora*. This suggests that the pigment of the Bermuda actinians is probably identical with Moseley's actiniochrome, but only a spectroscopic examination can determine this with certainty. The two species worked on by Moseley were subsequently investigated by MacMunn (1885 *c*) with the result the Moseley's actiniochrome was identified, but, in addition, a haematin-yielding pigment was isolated and given the same 'actiniohaematin'.<sup>2</sup> This pigment, which, it should be emphasized, is closely related to haemoglobin, performs a respiratory function, being capable of existing in a state both of oxidation and reduction. On finding these two pigments together in one animal MacMunn drew the conclusion that one is a respiratory substance (actiniohaematin), and that the other (actiniochrome) is purely for ornament. The most notable contribution of MacMunn, however, was his observation concerning the relation of *Zooxanthellae* ('yellow cells')

<sup>1</sup> MacMunn (1885 *c*, p. 643). alcohol, ether, chloroform, and carbon bisulphide.

<sup>2</sup> This pigment, on treatment with a metallic hydroxide and sodium sulphide, gives haemochromogen which on oxidation gives haematin.

to the respiratory pigment. He found that in actinians which were not infected with Zooxanthellae a respiratory pigment is present, but that in forms which are 'packed with "yellow cells"' the pigment had ceased to perform a respiratory function. The most striking case which he observed was that of *Bunodes balli*, in which occurs a facultative association between itself and the Zooxanthellae: the larger variety of that species has many Zooxanthellae and, as a result, is almost without trace of any of the pigments common among other forms; the smaller variety of the same species is uninfected, and as a result possesses a respiratory pigment. The results of MacMunn's work are briefly as follows: (1) that a respiratory pigment is present in most actinians; (2) the pigment is not a carrier of oxygen, but serves simply to store oxygen in the tissue which is subsequently to use it; (3) in those actinians in which yellow cells are present the chlorophyllous pigment of these organisms seems to replace the respiratory pigment; (4) besides this respiratory substance there are other pigments (such as Moseley's actiniochrome) which serve for decoration.<sup>1</sup>

In a recent paper on *Actinia equina* and *Anemonia sulcata* Elmhirst and Sharpe (1920) record several observations which are not in agreement with those of MacMunn. They find that the non-haematin pigment, instead of being purely ornamental, produces oxygen, possibly by photosynthesis. However, they report the presence of Zooxanthellae and fail also to find any haematin derivative, which accords with the results of MacMunn. In addition, these authors hold that the intensity of colour in *A. equina* varies with exposure to light, and the pigment, therefore, functions as a light screen. Certain observations made in the course of the present study support this latter conclusion: when *A. bermudensis* is kept in the dark (three days) the animal loses its deep red tinge and acquires a brownish-red shade. Conversely, if an individual is exposed to direct sunlight, its colour changes to a brilliant carmine.

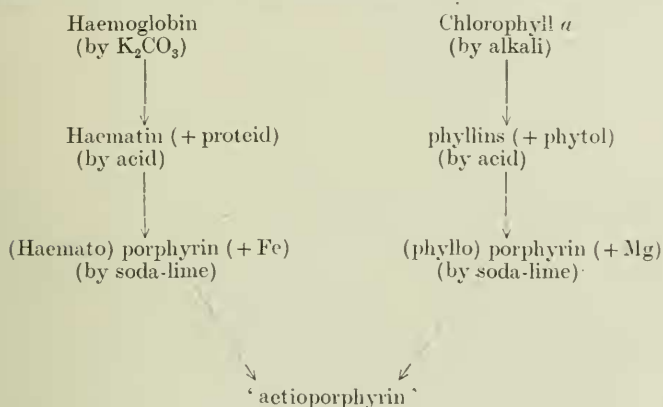
<sup>1</sup> Quoted also by Griffiths (1892); an excellent description of MacMunn's work will be found in Griffiths' book, especially in Chapter VIII.

Having considered the physical and chemical aspects of the actinian pigments, what deductions can be drawn as to their origin?

It is clear, inasmuch as there is no internal circulatory system between the ectoderm and entoderm, that the pigment must be manufactured from substances of the outside world which come actually into contact with the individual cells. Likewise it is evident that these substances are absorbed from within, carried, that is, in the chylaqueous fluid, since the ectoderm of an anemone serves for protection rather than for absorption. It is highly improbable that the pigment comes directly as food (as Crozier (1917) holds for a species of polyclad and Poulton (1893) for certain insects); if that were true, it should exist in solution in the gastrovascular fluid; but this seems definitely not to be the case (MacMunn). The more reasonable hypothesis, it seems to me, is that the cells containing the colour themselves synthesize the pigment from certain food substances. This means that in the absence of a blood-system each actinian cell has to elaborate its own pigment.

Little is known concerning the nature of the food from which the cells manufacture pigment. Various speculations, however, have been made on this point, particularly in the case of the insects. In this class of animals, as Poulton (1893) has shown, the pigment of the body appears to be a modified chlorophyll. That such a condition should obtain among the actinians seems at first impossible. Many actinians live in an obligate association with Zooxanthellae, an association in which the anemone is probably parasitic upon chlorophyllous cells (Fulton, 1921 *a*); that is, in times of starvation they turn upon the cells from which they possibly receive nourishment (by photosynthesis) and engulf them. Also in many actinians the 'yellow cells' are lodged directly in the tissue of the ectoderm and entoderm (Hertwig, 1883). It is a matter of common knowledge, too, that actinians feed upon pelagic forms which contain chlorophyll. From these facts it is evident that actinians use chlorophyll as food. As has already (p. 348)

been stated MacMunn (1885 *c*) demonstrated in many actinians the presence of a haematin-yielding pigment, which was designated as actiniohaematin. Recent investigations have shown that haemoglobin and chlorophyll are similar chemically, each having as a base a substance known as a porphyrin. This derivative is composed of four pyrrol groups in complex linkage. The exact similarity which exists between haemoglobin and chlorophyll may be shown by the following :<sup>1</sup>



Thus haemoglobin, by the loss of a proteid (globin) and its iron, forms a porphyrin: in the same way chlorophyll *a*, by the loss of phytol and its magnesium, forms several porphyrins, one of which (phyllo) is spectroscopically and chemically very closely related to haematoporphyrin; both phylloporphyrin and haematoporphyrin by the action of soda-lime give the same substance, actioporphyrin. From this it seems evident that there is no *a priori* reason for assuming that the tissues of actinians could not convert chlorophyll into such a substance as actiniohaematin, which is closely related to haemoglobin. The obvious objection is that exchange of metals (iron for magnesium) would make such a transformation impossible. But one should recall that it is a far more simple

<sup>1</sup> The best discussion in English of the chemistry of haematoporphyrin and chlorophyll is that of Plimmer (1915); Bayliss (1918) is good, but the standard work is that of Willstätter and Stoll (1913).

process to drop off an atom of magnesium and add one of iron than it is to build up an enormously complex molecule such as the porphyrins present.

If such a view is capable of experimental proof it will have an important bearing upon the phylogenetic origin of animal pigments; it will give fair indication that many animal pigments were derived originally from plant chlorophyll as the result of some symbiotic association (perhaps for the purpose of facilitating respiration) of an animal with a chlorophyll-bearing organism—a condition probably similar to that found to-day among the sponges and certain of the Protozoa. On the basis of this theory it is interesting to speculate concerning the origin of haemoglobin in the higher animals. Is it not possible, for instance, that our blood-pigment is derived from the chlorophyllous substances which are taken in as food, a condition not unlike that which the writer believes to exist in the coelenterates? The recent feeding experiments of Bürgi and his co-workers (1919) indicate that such is the case, for they give strong indication that the animal body is dependent upon chlorophyll for the building of haemoglobin; of three sets of anaemic rabbits, one was fed alone upon a chlorophyll diet, the other upon iron pills, and the last group upon a mixture of iron pills and greenstuffs. The anaemic condition of the first two groups was very slow to improve, whereas the animals in the last group within a short time lost all symptoms of anaemia and the haemoglobin content of their blood came back to normal. This means that chlorophyll with its four pyrrol groups is quite as necessary for the manufacture of haemoglobin as elemental iron. This conclusion is further substantiated by Grigoriew (1919), who has repeated Bürgi's feeding experiments with positive results. If this be true, methods can very well be devised to control the formation of haemoglobin in disease.

What, then, must be the conclusion as to the origin of pigment in the coelenterates? In the first place the chylaqueous fluid, which in function at least is the analogue of the blood of higher animals, carries to the tissue the components

from which it elaborates its pigment; the components, in addition, are probably highly-organized substances. The gastrovascular fluid derives these pigment-making substances from the chlorophylls which enter as food. The pigment, therefore, in addition to bearing a close relationship to haemoglobin, is probably itself derived from chlorophyll. This applies to MacMunn's actiniohaematin; and it will also be recalled that Elmhirst and Sharpe (1920) have shown that the non-haematin pigment of *A. equina* releases oxygen as a result of photosynthesis, which likewise suggests an intimate relation to chlorophyll.

#### 4. PLATYHELMINTHES.

Huxley (1877, p. 57), writing of the digestive cavity in the Coelenterata, remarked that the 'fluid which it contains represents blood'. Concerning the next higher group he states: 'In the Turbellaria, Trematoda, and Cestoidea, the lacunae of the mesoderm and the interstitial fluid of its tissues are the only representatives of a blood-vascular system.' The observation of Huxley is interesting, but it must be recalled that the mesodermal lacunae represent merely the morphological homologue of the blood-system;<sup>1</sup> the functional precursor of the vascular system, as in the coelenterates, is to be found in the gastrovascular cavity.

Pigmentation is common among the flat worms; many of the marine polyclads, in particular, are distinguished by a brilliant coloration. The only investigations concerning the pigment of the animals belonging to this class with which the writer is acquainted are those of Gamble and Keeble, and Crozier. The latter author (Crozier, 1917) has shown that the polyclads

<sup>1</sup> Though the supply of pigment-forming substances is undoubtedly given by the gastrovascular system, it is interesting to note that among the Rhadocœle Turbellaria the parenchyma (in which the lacunae are formed) is the seat of the body-pigment (Parker and Haswell, vol. i. p. 265). This seems to be the first instance in which the function of providing pigment has been taken over by the morphological fundament of the future blood-system.

commensal with the orange colonies of *Ecteinascidia turbinata* and the purple colonies of *Rhodozonia picta* are themselves orange and purple, respectively, and of a shade very similar to that of the animal with which they are commensal. On starvation (i.e. when removed from the colony of tunicates) these polyclads lose their colour, but when allowed to feed again with the tunicate colonies they regain their colour in a very short time. This, Crozier believes, is an example of a pigment which is formed directly from food, and it accounts for the colour being the same as that of the animal with which the polyclad is commensal. The writer has made certain other observations which in part support Crozier's conclusion. The colouring matter in the tunic of *Ecteinascidia turbinata* is made up of stellate orange chromatophores. Now, if the body-cavity in one of the polyclads recently taken from a colony of *Ecteinascidia* be observed under the microscope, not infrequently small pieces of orange pigment can be observed, many of which show clearly that they are portions of the chromatophores from the tunic. Owing to the great frailty of the polyclads, the fate of these small pieces of pigment could not be followed completely; as a result it was impossible to settle definitely whether the pigment was ingested bodily by the entodermal lining as Congo red is ingested by the young of *Convoluta roscoffensis* (Gamble and Keeble, 1903), or first went into solution. If the former assumption were true, it might be possible to raise a race of colourless individuals of this species (as Poulton, 1893, has done for certain of his insect larvae) by preventing their association with *Ecteinascidia*. The writer, however, is inclined toward the belief that the fragmentary pieces of chromatophore go into solution in the water-vascular system and are subsequently taken up by the cells which need them. The latter explanation, if correct, would accord with the fact that the pigment does not extend promiscuously over the body, but is found in definite and regular designs.

Among some Turbellarians (*Convoluta* and *Vortex*) the green or yellow colour is occasioned by the presence of symbiotic algae.



## 5. ECHINODERMATA.

It is generally accepted that the first phylogenetic appearance of a vascular system is to be found among the echinoderms. The animals of this phylum are provided with distinct organs of circulation consisting of two radiating canal systems (haemal and perihæmal), the most important of which arises from a ring surrounding the oral end of the digestive tube.

Although these vessels always contain a corpusculate fluid, it is certain that they are incapable of either peristalsis or of any other contractile manifestation. Also the sinus which accompanies the madreporic canal, while usually looked upon as a rudimentary heart, certainly performs no pumping function. Consequently serious doubt has arisen as to the correctness of looking upon the haemal and perihæmal systems of the echinoderms as true blood-vascular systems.

The corpusculate fluid of the haemal and perihæmal vessels is likewise found throughout the entire peritoneal cavity. 'The corpuscles are nucleated cells, which exhibit amoeboid movements; and the fluid so obviously represents the blood of higher animals, that I know not why the preposterous name of "chylaqueous fluid" should have been invented for that which is in no sense "chyle", though, like the other fluids of the living body, it contains a good deal of water' (Huxley, p. 480).

(a) The Sea-urchin *Tripneustes esculentus*  
Leske.

The red pigment cells are the most noticeable constituent of the body-fluid of this animal. With them are found non-pigmented cells, vibratile cells which are supposed to facilitate circulation, and, less frequently, yellow cells which are not unlike *Zooxanthellae*.<sup>1</sup> The red cells are closely packed with small granules; when protruding its pseudopodia, the cell first sends forth a thin, transparent lamella of hyalin ectoplasm,

<sup>1</sup> The most recent work on the body-fluid cells of sea-urchins is that of Kollmann (1908). He recognizes five types of cell.

and into this the round red granules subsequently flow. The conformation of the pseudopodia resembles closely that described by Goodrich (1919) for the coelomic corpuscles of *Asterias glacialis*. Thus (as first shown by Geddes, 1880) the cell is truly amoeboid, being able both to protrude and to withdraw its pseudopodia.

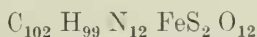
It seemed a curious fact that the cells within the body-fluid of the sea-urchin should be of identically the same colour as the pigment granules which give to the animal its characteristic coloration. Consequently an effort was made to determine whether or not the two pigments are identical. The same technique was employed as was made use of in settling the identity of the pigments from *A. bermudensis* and *C. passiflora* (p. 347), viz. that of testing their action in the presence of certain solvents, and the results seemed to indicate clearly that the two pigments are one and the same. In examining the external pigmentation, small pieces of the tube-feet were employed, since the behaviour of their coloured granules can be watched much more closely than can those of the spines. For testing the perivisceral fluid, fresh smears were used to which the reagents were added with a capillary pipette while under observation. Of the alcohols, amyl was the only one which dissolved the pigments, they being very readily soluble, however, in this reagent. Neither of the pigments were extracted by the lipochrome solvents, ether, chloroform, petroleum ether, or xylol. The colouring-matters are readily dissolved by 10/N solutions of the acids, dissolving with particularly great rapidity in valeric acid.<sup>1</sup> In the presence of alkalis both pigments were darkened, but not extracted.

After these experiments had been made, it came to the author's attention that Geddes, in a personal communication to Gangee (1880, p. 134), stated his belief that the pigment of the red amoeboid cells was identical (in *Echinus*) with that of the epidermal spines. This view resulted from a very thorough study of the body-fluid of sea-urchins (Geddes, 1880). Con-

<sup>1</sup> This again corroborates Crozier's (1916 *a*) conclusion that valeric is the most penetrating of all acids.

cerning the origin of these pigment cells Geddes and Prouho (1887) agree that the yellow cells<sup>1</sup> which are found in the body-fluid give rise to the red cells; they base their conclusion upon the fact that at times many intermediate stages between yellow and red cells are to be observed. In sea-urchins which had been weakened on the preceding day by the loss of perivisceral fluid, the writer also noticed intermediate stages between these two corpuscles.<sup>2</sup> The observations regarding the origin of the red cells are of importance, since they give direct evidence that the body-pigment arises in the blood-system.

Very little is known regarding the nature of the yellow cells from which the red ones arise. It is quite possible that they are chlorophyllous cells, as Geddes has suggested. But if this is the case, we have before us a phenomenon of great importance, since it would afford direct proof that a chlorophyllous substance gives rise to a haematin pigment. MacMunn (1883 *c*) has found chlorophyll in the integument and certain tissues of many invertebrates, including the echinoderms. In addition he (1883 *a*) has described the red pigment of echinoderms as being a definite chemical substance, which he has named 'echinochrome'. This pigment was believed both by him and by Griffiths to be respiratory in nature, but this has since been denied by Saint-Hilaire (1896), and more recently by McClendon (1912). Echinochrome has been analysed chemically by Griffiths (1897) with the following result:



On boiling, the pigment is converted into haematoporphyrin (p. 351) and haemochromogen, which shows that echinochrome is related to haemoglobin; the relationship, moreover, is probably close, since it has in the molecule both Fe and S, and also because it breaks down into haemochromogen, which

<sup>1</sup> Found most abundantly in *Doroecidaris papillate*. Arbacia, and particularly in *Spatangoidea*.

<sup>2</sup> In the study of the pigmented cells of *A. atra* (Fulton, 1921 *b*) a similar phenomenon was observed; in animals which previously had been weakened by the loss of blood many intermediate stages between the colourless cells and the pigmented corpuscles were observed.

is a reduction product of haemoglobin. It is interesting also to note that MacMunn (1883 *b*) has shown the presence of free haematoporphyrin in the tissues of echinoderms (especially in *Uraster rubens*) which, as he suggests, is probably an intermediate product in the formation of echinochrome.

From the preceding facts it is evident that in most echinoderms there are present simultaneously chlorophyll, haematoporphyrin, and echinochrome, the last being closely related to haemoglobin. If the diagram on p. 351 be consulted it will be observed that this represents almost the complete circle from chlorophyll to haemoglobin: thus we have chlorophyll, a porphyrin, and an iron-containing pigment which breaks down into a reduction product of haemoglobin. This evidence greatly emphasizes the hypothesis (p. 352) that many of the animal pigments, including haemoglobin, are derived originally from chlorophyll.

#### (*b*) Other Echinodermata.

The body-fluids of numerous other echinoderms possess coloured amoeboid cells whose pigment in many cases appears to be identical with that of the epidermis. The fact that the colour of these cells is similar to that of the body is in itself significant; in addition, however, there is frequently chemical evidence which tends also to establish their identity. To avoid tiresome repetition only a few of the more important examples from several of the classes of echinoderms will be given.

*Asteroidea*.—The amoebocytes from the coelomic fluid of starfish possess granules which vary in colour from yellow to a deep brown (Cuénot, 1901). It is noteworthy, moreover, that the cells with the darker granules are found in the animals with a deeper body-colour. This is particularly noticeable in some of the Bermuda starfish.

*Ophiuroidea*.—The only species examined was *Ophiocoma pumila*. In this form the body-fluid was marked by the presence of many yellowish-brown pigment cells which were closely allied in colour to that of the disc.

*Echinoidea*.—So far as the writer is aware, in every

species of echinoid whose blood-cells have been reported upon, the existence of a red, or red-brown pigment cell (such as was described for the Bermuda sea-urchin) has been noted; and a careful study of the epidermal pigment would undoubtedly reveal, as it did in *T. esculentus*, that it is identical with that of the coloured amoebocytes.

*Holothuroidea*.—The paper of MacMunn (1889) on animal chromatology contains many valuable observations concerning the pigments of *Holothuria nigra*. He has shown the presence of a red lipochrome in the 'blood' of this form and has found an identical substance in the integument. He identified the same pigment in the digestive gland and concluded that it 'is built up in the digestive gland and carried in the blood current to be deposited in other parts of the body, though what its rôle may be when deposited there it is difficult to say'. The fact that this pigment is found in the digestive gland is of particular importance, and should be borne in mind when the pigmentation of the crustaceans is considered. In passing, it seems worth while to note that this red lipochrome described by MacMunn is the one which Harvey (1915) found in the testis of *Stichopus ananas*, on which he has performed an interesting series of permeability experiments, using the lipochrome within the cells as an indicator.<sup>1</sup> The writer has observed amoebocytes with brown pigment granules in the body-fluid of *Holothuria surinamensis*—pigment granules which are similar to those of the epidermis. In addition Hérouard (1889) and Cuénot (1891) have reported brilliant brown and yellow amoebocytes for many holothurians common along the French coast. One holothurian (*Cucumaria planci*, Marenz.) is distinguished by the presence of haemoglobin in the cells of the perivisceral cavity. Kollmann (1908, p. 188) endeavoured to find the origin of these cells, but was unsuccessful.<sup>2</sup>

<sup>1</sup> The substance turns blue in acid (MacMunn, 1889; Harvey, 1915).

<sup>2</sup> Haemoglobin is found also in the ophiuroid, *Ophiaetis virens* (Foettinger, 1880); derivatives of haemoglobin (actiniohaematin), moreover, are found in many actinians.

## PART II. THE PIGMENTS OF ANIMALS WHICH HAVE A BLOOD-VASCULAR SYSTEM.

### 1. INTRODUCTION.

In the first half of this study consideration was given to those invertebrates which possess no blood-vascular system, and the general conclusion was reached that the body-pigments, in the absence of such a system, are deposited in the organism by its nutritive fluids. It was concluded also that the materials used in the production of pigment are derived from certain substances—usually chlorophylloid—which are taken in as food. It seemed, therefore, that in the lower invertebrates the body-fluids (which serve the nutritional function of the blood of higher animals) serve also to supply the organism with the substances from which it elaborates its pigment. In echinoderms, for example, the pigment is formed in the perivisceral fluid, and is subsequently carried to the epidermal regions. As a result of such observations one might reasonably expect the blood of the higher animals also to furnish the supply of materials for the body-pigments. The writer, in consequence, has examined the evidence to determine whether such a deduction is justifiable; the results of the investigation are presented in the present half of the paper.

### 2. NEMERTINA.

Though the nemertean worms possess certain annelid affinities (nephridia, &c.), the balance of morphological evidence places them close to the Turbellaria. In one very important characteristic, however, they are distinct from all of the Platyhelminths, and that is in the possession of a well-developed blood-vascular system which is entirely closed. The vessels originate from a fusion of the spaces which arise in the mesoblast. The circulatory fluid contains flat, nucleated corpuscles, and it is propelled through the body, probably by bodily contractions, though there is some evidence of vascular peristalsis. Few investigations have been made upon the

pigmentation of nemerteans; one form, *Polia sanguirubra*, however, is distinguished by the presence of haemoglobin in the blood-cells (Hubrecht, 1874). Previously Lankester (1872) had found haemoglobin dissolved in the plasma of this form. In *Cerebratulus urticans* (Hubrecht and Shipley, 1911) haemoglobin is found in the wandering body-fluid cells. It is reasonably certain, therefore, that the red colour of certain nemerteans is due to the presence of haemoglobin in the blood and in the body-fluids. The origin of the haemoglobin is not known, but it is interesting to note that the food of these worms is largely chlorophyllous.

### 3. MOLLUSCA.

Among the molluscs the most highly-coloured groups are the opisthobranchs and the cephalopods, and to these brief consideration will be given.

#### (a) The Opisthobranchs.

Crozier (1914, 1916 *a*, and 1916 *d*) found that the coloured substance in the mantle of the nudibranch *Chromodoris zebra* is very sensitive to the presence of acids and alkalis, being blue in its natural state (alkaline) and pink in acid. He (1916 *b*) observed further that the blood-cells contain a pigment which is similar in colour to the mantle pigment and likewise turns pink in the presence of acid. An examination of the absorption spectra of the two coloured substances showed that they are identical. Thus the pigment of the blood is the same as the pigment which is responsible for the external coloration.<sup>1</sup> Inasmuch as it has been shown that the pigment cells in the blood of other forms—echinoderms (Part I of this paper, p. 357) and Tunicates (Fulton, 1921 *b*)—arise while in circulation, it is not unreasonable to assume, until further evidence is afforded, that the blood-pigment of *C. zebra* is also synthesized by the moving blood.

<sup>1</sup> The writer attempted to secure specimens of *C. zebra* when in Bermuda in order to find the origin of the pigment cells of the blood, but owing to the scarcity of this species during the summer months none were obtained.

Little is known concerning the chemical nature of the pigments of opisthobranch molluscs ; however, certain observations of Crozier and others are of interest. Crozier reported the presence of manganese in the pigment of *C. zebra*, though he did not believe that the manganese facilitated respiration. Paladino (1908) detected both magnesium and iron in the pigment of *Aplysia punctata*.<sup>1</sup> It appears to the writer that Paladino's observations are of particular importance in their relation to the origin of animal pigments. In the first part of this paper, evidence has been given to show that the pigment haemoglobin is derived from chlorophyll. The chief objection to such a contention is the difficulty of exchanging the magnesium of chlorophyll for the iron of haemoglobin. It would seem from Paladino's work that the coloured substance of *A. punctata* is a pigment which is intermediate between chlorophyll and haemoglobin since it possesses both magnesium and iron. It probably is not entirely justifiable to draw any conclusion until more is known concerning the chemical nature of the molecule to which the iron and magnesium are attached. However, the pigment showed absorption bands which are similar in some respects to those of haemoglobin ; moreover, nitrogen was detected in the molecule. Both of these facts bear evidence that the pigment of *A. punctata* possesses the same chemical base as haemoglobin.

The blood of a closely-allied form, *Aplysia depilans*, has a distinct rose colour, due to the presence of an albuminoid (Cuénot, 1890) which is precipitated by alcohol and acids. The coloration of the body is due in part to the presence of this substance in the epidermal regions.

#### (b) The Cephalopods.

Among the earlier observations on the blood of cephalopods were those of Rouget (1859), who described coloured corpuscles

<sup>1</sup> MacMunn (1899 *b*) has made a spectroscopic investigation of the pigments of *Aplysia punctata*. He also found (as did Crozier and Paladino) that the pigment is very sensitive to a change from alkalinity to acidity.



in an octopus, and of Bert (1867), who gave an interesting description of the epidermal circulation. Rabuteau and Papillon (1873) obtained a pigment from *Octopus vulgaris* which failed to give absorption bands. The classic researches on this form, however, are those of Fredericq (1878), who was the first to demonstrate the presence of a respiratory pigment (on haemocyanin, see below) in the blood. In addition to the haemocyanin he found in the blood a red lipochrome. Though there is no direct proof (as there is in the case of the red lipochrome found in the blood of crustaceans) that the substance is identical with the red pigment in the chromatophores, it is highly probable that such is the case. The colour itself strongly suggests the similarity, while the fact that similar pigments are found in the blood of other animals as well as in their chromatophores, gives added evidence that the two pigments are identical. The best description of the chromatophores in *Octopus* is that of Cowdry (1911).

The inky fluid of the cuttle-fish *Sepia officinalis* is a pigment which is chemically identical with certain melanins of the higher animals (Piettre, 1911 *b*). Though melanin-like substances have been reported in gorgonian stems and in certain mollusc shells, their identity with vertebrate melanins has never been proved. Consequently it is a very curious fact that the invertebrate which possesses the most highly organized nervous system should also possess melanin, which in the higher animals is so intimately connected with the nervous system. The question of the origin of melanin and of its chemical constitution will be considered in a later section.

### (c) Other Mollusca.

Many detailed investigations have been made on the respiratory pigments of the mollusca, but since these pigments seldom function in the production of coloration, they will not be considered in detail.

**Haemocyanin.**—The substance responsible for the blue colour of the blood of certain mollusca was first isolated and described by Fredericq (1878). Haemocyanin has since been

found in representatives of every class of mollusc save the Amphineura,<sup>1</sup> and it is also common among the crustaceans and the arachnids. Recently the chemical and physiological properties of haemocyanin have been extensively investigated by Alsberg and Clark (1914), who find that the pigment lacks many of the remarkable properties of haemoglobin; particularly to be noted is its small binding power for oxygen.

**Pinnaglobin.**—The blood of the lamellibranch *Pinna squamosa* is distinguished by a respiratory pigment which contains manganese. This metal is linked to a protein which contains the usual elements of blood pigments, viz. C, H, N, S and O<sub>2</sub>, and these in proportions similar to the corresponding elements of haemoglobin (Griffiths, 1897).

**Haemoglobin.**—The colour of the 'blood-clam' (*Arca*) is due to the presence of haemoglobin in the blood; the pigment is found also in other Lamellibranchs: *Solen legumen*, and *Cardita*, and in the gastropod *Planorbis* (Lankester, 1872; Leitch, 1916).

Chlorophyll has been detected in many of the Mollusca. The colour of the green oyster is due to an algal infection (Lankester, 1886). Enterochlorophyll has been described by MacMunn (1883 *c*, 1885 *b*) as existing in the liver of many crustaceans and molluscs. For further discussion of enterochlorophyll, see below, p. 369.

#### 4. THE ANNELIDA.

One of the most interesting characteristics of the coloration in annelids is the fact that the coloured substances found in this phylum are usually respiratory pigments; few of the coloured substances serve alone for ornament. The segmented worms have two circulatory fluids: the perivisceral (coelomic)

<sup>1</sup> The chitons possess a non-metallic respiratory pigment,  $\beta$ -achroglobin, which was described by Griffiths (1897). In addition to this pigment a yellow lipochrome is present in the blood, which is more common in the female of the species than in the male, and is thus responsible for the deep yellow colour which the female chiton assumes during periods of reproduction.

and the pseudohaemal, or blood-vascular fluid. The fluid of the coelom contains corpuscles, and carries food substances derived from the intestine. The blood, on the other hand, functions in respiration and probably has little to do with the transportation of food. The respiratory pigments, therefore, are found in greatest abundance in the blood-vascular system, though they are sometimes met with in coelomic fluid.

There are four varieties of pigment found in annelid worms : haemoglobin, chlorocruorin, chlorophyll, and the lipochromes.

**Haemoglobin.**—In the more primitive annelids, haemoglobin is found dissolved in the blood plasma, the corpuscles themselves being colourless and serving as phagocytes (most chaetopods and Hirudineae; Lankester, 1872). In the Capitellidae and in Glyceridae the haemoglobin is packed in individual cells.<sup>1</sup> Much more extraordinary, however, is the occurrence of haemoglobin in the nervous chain of *Aphrodite aculeata*, and as Lankester (1872, p. 79) remarks it is difficult to account for its appearance there as 'we have no knowledge that this Annelid is remarkable for nervous energy'.

Annelid haemoglobin (particularly that of *Lumbricus terrestris*) is almost identical with the respiratory pigment of vertebrates. Griffiths (1892, p. 147) made a detailed chemical analysis of the haemoglobin of *Lumbricus* and found that the only considerable discrepancy between it and the haemoglobin of a dog lay in the amount of iron present, there being slightly less iron in the pigment of the annelid.

Concerning the differences found between the various haemoglobins, a statement which Griffiths (1897, p. 101) makes in another work might very aptly be quoted. 'If species have been modified in the course of long eras, the haemoglobins—though fulfilling all the conditions of a true chemical compound—must have become modified step by step with the species. . . . Hence we see that the conception of evolution must necessarily find a place in chemistry, not merely as

<sup>1</sup> In many text-books—Parker and Haswell, Hegner, &c.—it is stated that the haemoglobin of annelid blood is found dissolved in the plasma. Though true for certain species it is quite incorrect as a general statement.

regards the elements, but as regards the formation of highly complicated compounds.' Though the earlier writers considered that there were no spectroscopic variations between the haemoglobins of various animals, more recent work has shown that there are minute differences even between the spectral bands of ethiopian haemoglobin and that of the white races. Recently Vlès (1919) made a careful investigation of the haemoglobin of *Arenicola piscatorum*, Lk. and of *Marphysa sanguinea*, Quatref., and has found certain pronounced differences between their spectra and that of vertebrates.

With such facts as these one cannot avoid agreement with Griffith's contention that haemoglobin is the product of a long and complicated phylogenetic development. Sorby (1876), in a paper on the evolution of haemoglobin, held that the substance had its beginning as a bile pigment (non-respiratory) such as exists in *Helix aspersa*. The next step was found in the haemoglobin-like pigment of *Planorbis*, which is respiratory. The final stage is seen in the concentration of haemoglobin into individual cells, a condition such as one finds first in the blood corpuscles of *Gephyrea*, and later in the vertebrate erythrocytes.

Reichert and Brown (1909), in their monumental work on the crystallography of the haemoglobins, have urged, since there is a characteristic crystal form for the haemoglobin of every species, that the haemoglobin of each species is chemically different from that of every other, and that a specific crystal form is an expression of a specific chemical molecule. But, 'it is well known that crystal-habit is modified by the alterations of the medium from which the crystals are deposited', and since it is a matter of common knowledge, particularly in the light of modern bacteriology, that the blood plasma of animals differs profoundly between individuals (even of the same species), and since the haemoglobin crystals of each species studied by Reichert and Brown were deposited from a different medium, 'it is not improbable that the observed differences in the crystals are attributable to these known differences in the media in which they were formed' (Robert-

son, 1920). This, however, does not in any way destroy the value of their work, which is a most fundamental contribution to our knowledge of the crystal structure of proteins.

**Chlorocruorin.**—This pigment, though green in colour, is closely allied to haemoglobin. The substance was noted by Milne-Edwards (1838), but was first isolated by Lankester (1869) from *Sabella ventrilabrum* and *Siphonostoma*. MacMunn (1883 *a*) subsequently identified it in *Serpula contortuplicata*.<sup>1</sup> Though the absorption bands of chlorocruorin present a striking similarity to that of haemoglobin, the green pigment cannot be broken down into any of the decomposition products of the red pigment. Chlorocruorin, however, is definitely respiratory and is capable of existing in two states of oxidation, oxychlorocruorin and reduced chlorocruorin. The pigment is found both in the blood and in the epidermis, and the animals possessing chlorocruorin owe their colour almost entirely to its presence.

**Chlorophyll.**—The only authentic example of a chlorophyllous pigment existing in annelids is that of *Bonellia viridis*. Sorby (1875 *b*) showed that this pigment gave absorption bands which were practically identical with chlorophyll, but Geddes (1882) failed to find any evolution of oxygen in the tissues of *Bonellia* when it was exposed to strong sunlight. Krukenberg likewise obtained negative results (Geddes). However this may be, it is most probable that bonellein is a derivative of chlorophyll, particularly in view of the recent investigations of Hans Przibram (1913), which have demonstrated striking spectroscopic similarities between the two pigments.

**Lipochromes.**—The blood of *Arenicola piscatorum* has, in addition to haemoglobin, certain lipochromes, some of which give absorption bands (MacMunn, 1883 *a*, 1889). MacMunn, in addition, found that a lipochrome present in the digestive tract was found also in the epidermis. Fauvel (1899)

<sup>1</sup> It is a curious fact that the pigment of *Serpula*, though spectroscopically identical with that of *Sabella*, is red in colour. Griffiths (1897) believes that the two pigments are isomeric.

has likewise reported the presence of a yellow lipochrome in the epidermis of *Arenicola*. Inasmuch as Racovitza (1895) has shown that the amoebocytes of many polychaet annelids (including *Arenicola*) carry fatty pigments from the blood and deposit them in the epidermis, the unavoidable conclusion remains that the fatty pigments of *Arenicola* are derived from food, and transported to the epidermis by the blood-system. It is worthy of note also that many of the lipochromes are closely allied to carotin, which means that they are derived from chlorophyllous food substances. Further consideration of carotin will be given in the following section (p. 373).

## 5. ARTHROPODA.

In the phylum Arthropoda, not only is the blood-system well developed, but there are present, as in the cephalopods, large chromatophores which facilitate colour change. A vast amount of work has been done upon the pigments of the arthropods, and since it has been reviewed very completely by Fuchs (1914, crustaceans), and Biedermann (1914, insects), the writer will limit himself to a discussion of the origin of the pigments in this phylum.

### (a) Crustacea.

The presence of chromatophores in the carapace of crustaceans was first noted by Focillon (1851). The classic work on crustacean pigment, however, is that of Gamble and Keeble (1900) on the colour phases of *Hippolyte varians*, in which the structure and activities of the chromatophores are described in great detail. The structure of the chromatophores from other species of Crustacea has been investigated more recently by Franz (1910). The pigments which have been found in the blood of crustaceans are very nearly the same as those found in the molluscs, viz. chlorophyll (enterochlorophyll), lipochromes, carotin, haemoglobin,<sup>1</sup> and haemocyanin. As the

<sup>1</sup> Haemoglobin has been detected in several crustaceans by Regnard et Blanchard (1883).

copper-containing pigment of the crustaceans is identical with that of the molluscs, its further consideration is unnecessary.

*Enterochlorophyll*.—MacMunn (1883 *c*) discovered that a pigment which was spectroscopically identical with plant chlorophyll occurred in the liver of many molluscs and crustaceans. He found that this pigment—which he named *enterochlorophyll*—occurs dissolved in oil globules or in granular form, but sometimes even in the protoplasm of the secretory cells of the liver. For a long time MacMunn believed that the occurrence of *enterochlorophyll* in the livers of invertebrates demonstrated that animals are capable of synthesizing chlorophyll. Finally, however, he was forced to abandon this position when he (MacMunn, 1899 *a*) found that *enterochlorophyll* is present in the intestine and blood of the forms in whose livers it also occurs. He was thus forced to admit that *enterochlorophyll* enters the animal as food and is stored in the liver. In his own words (p. 438): ‘I have been forced, I must confess against my inclination, to believe that *enterochlorophyll* is a pigment which primarily has been taken up from the intestine dissolved in a fatty medium, and is carried either by leucocytes, or in some other way to be deposited with this fat, and perhaps other reserve products, in the gastric gland. Whether it is utilized for the production of other pigments or not is a question for future investigation. That it is a chlorophyll derivative I now believe to be proved.’

The same conclusion was reached by Dastre (1899, p. 120), who states: ‘La chlorophylle hépatique n’est pas un produit animal fabriqué par le foie: c’est une chlorophylle végétale, venant des aliments, fixée seulement et conservée d’une façon remarquable dans le tissu hépatique.’ Further investigations were made by Dastre and Floresco (1898, 1899 *a*, and 1899 *b*) whose conclusions were the same. What is the significance of the occurrence of chlorophyll in the livers of crustaceans and molluscs?

The most obvious fact is that it is a food substance stored there for future use. But of even greater importance is the fact that chlorophyll is shown by these observations to be

capable of absorption into the animal body without being materially changed in the process. Hence if animals employ chlorophyll in building up their pigments, they have it on hand unchanged by the processes of digestion. This conclusion is strongly reinforced by more recent work. Dhéré et Vegezzi (1916) have shown that all of the chlorophylloid pigments pass without change into the liver of *Helix pomatia*. But the more important work is that of L. S. Palmer (1915, 1916), who has shown that certain plant pigments are absorbed from the intestine into the blood of a cow and from there pass unchanged into the milk. This investigation will be discussed in greater detail under carotin (p. 373). One is reminded at this point of the observations of Moglia (1910), that the depth of coloration in certain gastropods decreases in the winter, due as he believes to a lack of food, and also of M. E. Johnson's (1913) contention that *Rana* depends for its colour, not upon the amount of nutrition but upon being nourished by food substances which are properly pigmented.

MacMunn (1883 c) has shown that in certain molluscs—as *Patella*—a haematin is present in the liver in addition to enterochlorophyll, and there is every probability he adds, that the haematin finds its origin in that tissue. Though the haematin might have arisen independently of the chlorophyll in the liver, the more logical assumption (in view of the chemical similarities between chlorophyll and haematin, alluded to in Part I of this study) is that it is derived from the chlorophyll. As with the origin of echinochrome, the origin of molluscan haematin is highly uncertain, but the hypothesis just presented is interesting and certainly merits further investigation.

From the observations of Paladino (1910) one is led to believe that haematin is present not only in molluscs but likewise in the livers of certain crustaceans. Apparently unacquainted with MacMunn's work Paladino has reported the presence of a water-soluble ferruginous pigment in the livers of several crayfish. In addition, he finds a yellow lipochrome (probably carotin) which contains no iron. The iron-containing pigment which he finds is undoubtedly either haematin or a derivative.



**Lipochromes.**—Halliburton (1885), in an investigation of the blood of decapod Crustacea, found a red lipochrome present in addition to haemocyanin, which had been described previously. He extracted the red substance by first precipitating the proteins of the blood with alcohol and then extracting the red pigment with ether. He noted also that the amount of the red pigment varied in different specimens, but was unable to find an explanation for the fluctuation. In the same year MacMunn (1885 *a*) investigated Halliburton's red lipochrome and concluded that it was identical with the pigment of the chromatophores in the exoskeleton. Several years later Miss Newbigin (1897) reinvestigated the question. She corroborated MacMunn's results, but in addition found evidence that the red lipochrome was derived from the yellow pigment (probably the carotin contained in the enterochlorophyll; see below, p. 373) of the liver. Consequently, if Miss Newbigin's results are reliable, one would trace the origin and development of the red pigment in the chromatophores of crustaceans as follows: chlorophyllous substances enter the body as food; they are absorbed by the intestine and stored by the liver, in which tissue they probably undergo slight modification. The carotin contained in the chlorophyll of the liver, when called upon, is transformed into a red pigment substance—a lipochrome—and transported by the blood to the epidermis, where it is used in building up the chromatophore. The recent analyses of Verne (1920) have shown that the red pigment of the decapod Crustacea is a hydrocarbon identical chemically and spectroscopically with vegetable carotin, and possess the same empirical formula ( $C_{40}H_{56}$ ). This investigation removes any doubt which may have existed regarding the identity of the two pigments.

The fact that the red lipochrome present in the blood is subject to great fluctuation (Halliburton), is possibly to be explained by the moulting period of the animal. Immediately after the moult there is great demand for pigment to build up new chromatophores, which results in a diminution of the amount present in the blood. In this connexion the recent

observation of Paul and Sharpe (1919) is of interest. They have shown that immediately before the moult the contents of the liver greatly increase in quantity, and after the moult the amount returns to normal. This would certainly affect the amount of pigment free in the blood. G. W. Smith (1913) has called attention to the fact that the amount of red pigment (which he called 'tetronerythrin') varies in the female (crab) during periods of reproduction, being most abundant shortly before the eggs are produced.

Before proceeding to the consideration of carotin a word should be said concerning the red pigment, tetronerythrin. This substance was first described by Wurm (1871), who reported that it existed in several animals. Mérejkowski (1881) reinvestigated the pigment, and came to the conclusion that it was widely distributed throughout the animal kingdom, since he found it in every phylum. Mérejkowski's criteria for tetronerythrin (also known as zoerythrin) are as follows: the substance must be (1) insoluble in water; (2) soluble both in alcohol and ether; (3) blue in acid; (4) red in carbon disulphide; and (5) decolorized by light. Both Mérejkowski and Gautier held the pigment to be respiratory. In a later paper, Mérejkowski (Krukenberg) reported that he had demonstrated zoerythrin in 117 species. Krukenberg<sup>1</sup> found it also on the eye-lids of birds and in their feathers. MacMunn (1889), however, showed that a number of different pigment substances would respond to the tests used by Mérejkowski.

The substance which responds to the test for tetronerythrin in crustaceans is the red lipochrome described by Halliburton (Halliburton, 1885; MacMunn, 1889), and, as tetronerythrin, it has been extracted both from the epidermis and from the blood; this further substantiates the conclusion reached above, that the red lipochrome of the blood is identical with that of the chromatophores. In addition, MacMunn also held that tetronerythrin arises in the liver.

**Carotin.**—This substance is a yellow pigment found in

<sup>1</sup> Vergleich. physiol. Studien, Abth. 5, and (2. Reihe) Abth. 1, p. 151; Abth. 3, p. 128.

association with chlorophyll. It was first isolated from carrots (sometimes spelled 'carrotin'), from which it received its name.

Willstätter und Meig (1907) have shown that carotin is a crystalline unsaturated hydrocarbon ( $C_{40} H_{56}$ ) melting at  $174^{\circ}$ . It is of wide occurrence not only in the vegetable, but also in the animal kingdom, being found in the blood sera of most birds and mammals (Schunck, 1903; Palmer, 1915, 1916; and Hymans van den Bergh und Miller, 1920), and in mammalian milk; <sup>1</sup> the yellow pigment of butter fat likewise is composed largely of carotin (Steenboch, Sell, and Buell, 1921). It is also found in mammalian ovaries; however, the 'lutein' of the corporea lutea themselves is isomeric with xanthophyll, the oxide of carotin. The colored substance of egg-yolk is made up largely of xanthophyll, though it too contains some carotin (Palmer and Kempster, 1919 *a*, *b*, and *c*); carotin also is sometimes present in human urine, appearing there after carotin has been ingested (Hess and Meyers, 1919), and it has been isolated from gall-stones (Plimmer, 1915, p. 532). But of much greater interest is the fact that carotin is present abundantly in mammalian nerve-cells (Dolley and Guthrie, 1919 *a* and *b*), and in the fovea centralis of the human eye. Also the yellow pigment cells (xantholeucophores) in the epidermis of *Fundulus* contain carotin, but what concerns us more immediately is its occurrence in the chromatophores of crustaceans.

Blanchard (1890) made it evident that the epidermal pigment of the copepod *Diaptomus lacillifer* contains a large percentage of carotin. Keeble and Gamble (1902, 1904, and 1905) have corroborated this result, finding that the chromatophores of many other crustaceans also possess the pigment. They noted, too, that mobile fat globules are usually to be found in the branching pigment cells. It has been shown by Kohl (1902) that carotin was capable of photosynthesizing fats. With this fact before them, Keeble and Gamble made

<sup>1</sup> Palmer and Eckles (1914) have shown that the amount of carotin and xanthophyll in milk (human and cow) is in exact proportion to the amount of green food consumed.

an effort to find the origin of the fat globules in the crustacean chromatophores. The investigation proved that the carotin present in these cells produces the fat globules by photosynthesis. The work was carried out on *Hippolyte varians*, and the experiments were briefly as follows:<sup>1</sup> when *Hippolyte* is starved in the dark practically all of the oil disappears from the chromatophores; when starved in sunlight, however, the globules continue to exist as before; when a 'dark-starved' *Hippolyte* is exposed to sunlight (without feeding) the fat returns. Thus the crustaceans present a remarkable phenomenon: the plant pigment which has been eaten by the animal, is stored in the liver; later it is carried by the blood-system and deposited in the epidermal chromatophores, where it functions exactly as in the plants from which it was derived! The only other instance of such a phenomenon known to the writer is that (already referred to on p. 373, note) described by Palmer and Eckles (1913), who have shown that carotin and xanthophyll pass in the blood from the intestine to the mammary glands. It is extremely interesting, also, to note the recent observation of Findlay (1920), that carotin and xanthophyll are found in the mammalian adrenals, and are largely responsible for the colour of the glands.

#### (b) The Insecta.

In many ways the insects offer a more favourable condition for the study of animal pigments than any other group of animals. They are small, many are brilliantly coloured, and in addition the physiological processes of insects are on the whole less complicated than in other forms; moreover the more important pigments of insects are concentrated in the wings, which are thin and therefore well adapted for the purposes of observation.

The greater share of the work on the pigmentation of insects

<sup>1</sup> It is not within the scope of this paper to attempt to summarize Keeble and Gamble's results on the mechanism of colour change in this animal. They will be found in the papers cited above and in Gamble (1910); a more recent study of colour variation in crustaceans is that of Potts (1915).

has been carried on by the English investigator Poulton,<sup>1</sup> who found that the pigment of a large number of insects (in all stages of development) is a modified chlorophyll derived from the plant on which the animal feeds. The chlorophyllous substances are eaten, absorbed into the blood-system, and deposited in the regions of the body exhibiting pigmentation. The most valuable results in Poulton's work came from a spectroscopic examination of insect blood. His (1884) work on the pigment of *Sphinx ligustri* is interesting. The blood from the pupae of this form was examined and its spectrum recorded; then an extract was made of the *calceolaria* leaves upon which the larva feeds. When the spectrum of the extract was superimposed upon that of the blood, the bands were found to correspond in a very striking way. In Poulton's own words (p. 290):

'Considering the chemical change which must have taken place in the chlorophyll during digestion, rendering possible the passage of the walls of the digestive tract, and considering its chemical union with the proteid constituent of the blood, the resemblances of the spectra are very striking; in fact, the two spectra are far nearer each other than the ordinary spectrum of chlorophyll in alcoholic solution is to the unaltered chlorophyll of leaves'.

It was held by Poulton that the power of utilizing chlorophyll in building up pigments is an adaptation on the part of the insect which enables it to assume the colour of the leaves on which it feeds. As evidence for this he brought forward the fact that if larvae of *Trypoena pronuba* are fed respectively on green, brown, and white cabbage leaves, green, brown, and white larvae result according to the colour of the leaf on which they were fed. This result has since been corroborated by Levart et Conte (1902), who worked on *Attacus orizaba* and *Bombyx mori*.

Peterson (1913) has found that the chlorophyll which passes into the intestine of certain red caterpillars is modified into

<sup>1</sup> Poulton has written a large number of papers on this subject. The more important ones will be found in the bibliography under Poulton, 1884, 1889, 1892, and 1893.

a red substance (vanessa red), which is later absorbed and transported to the epithelium, where it is deposited and becomes the pigment of the wings and of the other body-parts. The unabsorbed portion of the red pigment is voided. The investigations of Gortner (1911 and 1912 *b*) on insect melanins are also of interest, since he has shown that they are sometimes formed from chlorophyllous substances (oxidizable chromogens) acted upon by the plant ferment, tyrosinase. More recently Schmidt (1919) has conducted similar investigations on insect melanins, and his results accord with those of Gortner.

It appears to the writer that the most significant part of all Poulton's work on insects is the demonstration that chlorophyll resists the digestive enzymes, and passes practically unchanged into the blood-system. Evidence has been cited to prove that this also is the case in the Mollusca and Crustacea, but the evidence is not conclusive (except in the case of carotin). In insects, however, there is undisputable proof, and it is of particular importance, since it shows that the theory that haemoglobin as a derivative of chlorophyll must not be ruled out by the fact that chlorophyll is incapable of passing through the digestive tract. In general, then, the epidermal pigments of Crustacea are derived from food and are carried to their destination by the blood-system.

## 6. THE TUNICATA.

The strongest evidence in support of the view that the epidermal pigments are deposited by the blood-system is to be derived from a study of the tunicates, where it is possible to predict that there will be found in the blood-stream pigment cells of a colour corresponding to that of the tunie. Moreover one can show that the pigmented corpuseles arise from colourless cells while they are in the circulation. The tunicate to be first considered is *Ascidia atra*.

### (a) *Ascidia atra*.

This species is distinguished from other tunicates, and in fact from almost all other animals, by the possession of an

exceptionally large variety (ten distinct types) of blood-cells. In the vascular fluid of this animal there are three kinds of highly-pigmented cells: green, orange, and blue; in addition there are four kinds of non-motile white corpuscles, and three other types which are distinctly amoeboid (Fulton, 1921 *b*). The writer has shown that all of the pigmented cells in *A. atra* arise directly in the blood-stream from unpigmented corpuscles. In the case of the green 'chromocyte' the metamorphosis from the colourless cell may be stimulated artificially and the complete process watched under the microscope. When an acid, preferably an organic acid of N/10 to N/20 strength, is added to a fresh smear of blood, all the non-motile colourless cells of one variety may be observed to take on a light shade of green, which gradually deepens: at the same time the cell fragments into large green lumps and finally assumes the characteristic form of the green pigment cell. Therefore, it may be inferred that in nature the green pigment cell arises from an unpigmented corpuscle as a result of an increase in acidity.

The orange and the blue cells also arise from unpigmented corpuscles, but in a slightly different manner. Various methods were employed in an effort artificially to stimulate the change from the colourless to the orange and to the blue cells. No response was secured from acids or bases, but it was observed in smears of blood taken from an animal which previously had been weakened by the loss of blood, that there occurred many intermediate stages between the unpigmented corpuscles and the orange cell or the blue cell. If one of the intermediate stages be carefully watched, under very favourable conditions, there is some indication that it gradually increases its depth of colour. With this evidence<sup>1</sup> the conclusion is unavoidable that all of the pigmented cells arise in the blood-stream directly from colourless ones.

The deep purple-blue colour of the tunic of *A. atra* is

<sup>1</sup> The details of the experiments and a more complete statement of the evidence for this conclusion will be found in the paper by Fulton (1921 *b*) on the blood of *Ascidia atra*.

caused by the presence of large blue pigment cells (Hecht, 1918 *a*; Crozier, 1916 *d*) containing spherical granules, which migrate from one part of the cell to another. The blue corpuscles of the blood-stream are like the pigment cells of the test in every detail of their structure. It remains, therefore, to establish the identity of these cells. In the first place, the presence in both cells of a very prominent vacuole is strong evidence in favour of their being identical. In testing the cells with various reagents another interesting resemblance was noted. It is known that calcium chloride in minute amounts has the power of greatly accelerating the activity of phagocytes (Hamburger, 1910 and 1916); it also causes a decided increase in the activity of the blue pigment cells both of the blood-stream and of the test. After a M/10 solution of  $\text{CaCl}_2$  has been added to a fresh smear of blood the blue cells, which in their quiescent state are nearly spherical, immediately send forth pseudopodia, and at the same time the blue granules within the corpuscles commence to move from one end of the cell to the other. In the test, however, the pigment cells, inasmuch as they are fixed within the substance of the test, cannot move their processes; there is, nevertheless, following the addition of  $\text{CaCl}_2$  to a section of the test, a decided activity on the part of the blue granules,<sup>1</sup> an activity which is similar to that displayed by the corresponding granules of the blood-cells. The conclusion, therefore, seems to be warranted that the two cells are identical, and consequently that the blue cells in the blood give rise to the pigment cells of the test.<sup>2</sup>

From these two kinds of evidence it is a reasonable conclusion that in *Ascidia atra* the coloration of the animal is eventually traceable to the colourless cells of the blood; for, as has been shown, the unpigmented cells give rise, while in circula-

<sup>1</sup> A description of a phenomenon of this kind is also to be found in Pizon's (1898, 1901) papers on the pigment granules of tunicates.

<sup>2</sup> Hecht (1918 *a*) states that when *A. atra* regenerates a portion of its test, there are a great many of the blue blood-cells in the area of the regenerating tissue.



tion, to the blue corpuscles ; these finally become lodged in the tunic, and in that way give rise to the surface pigmentation of the animal.

(b) Other Tunicates.

There are many other ascidians in the Bermuda waters which are highly coloured. In a cave on the west side of Agar's Island<sup>1</sup> five specimens of the brilliant red tunicate, *Microcosmus miniatus*, Verrill, were found. An examination of the blood revealed that its most prominent constituent was an amoeboid cell, containing many brilliant carmine-coloured granules, which was very similar to the pigment cell that colours the test. The animal from which the blood had been extracted was examined on the day following, and, as in *A. atra*, there were many intermediate stages between the colourless cells and the pigmented corpuscles. These observations confirmed in a substantial way those made upon *A. atra*.

Other species of ascidians have been examined and in every case the colour of the pigment cells in the test was duplicated by the coloured cells of the blood. The colonial form, *Ecteinascidia turbinata*, Herdman, which is brilliant orange in colour, has as its only coloured cell in the blood-stream a corpuscle possessing orange granules.

(c) Discussion.

Concerning the origin of the pigments in the blood-stream of ascidians, no definite statement can be made. It has been observed that the pigment cells arise while in circulation from unpigmented corpuscles. Just what is the process involved in that colour change it is difficult to explain. Griffiths (1897) has described a colourless respiratory proteid ( $\gamma$ -achroglobin) in the blood of ascidians, and the chromogen of *Phallusia* has been stated by Henze (1911, 1912) to be a proteid in combination with the element vanadium. It is possible, therefore, that the change from the colourless cell to the 'chromocyte'

<sup>1</sup> Where the laboratory is situated.

is occasioned by the chemical union of the proteid with the metal. This seems extremely unlikely, however, since vanadium does not exist in the blood in its elemental state—the differences in colour of the corpuscles being due to vanadium in different states of oxidation—and also because the vanadium probably has the rôle of catalyst in the respiratory phenomena of ascidians (Fulton, 1921 *c*). A more likely explanation is that in the colourless antecedents of the pigment cells there exists some colourless vanadium compound which, either on oxidation or reduction, is converted into one of the coloured oxides of that metal.

#### 7. DISCUSSION—PIGMENTATION OF VERTEBRATES.

The results obtained in a study of the pigmentation of invertebrates cannot be entirely without application to the problem of coloration in vertebrates. Particularly does this seem true in view of the question regarding the origin of melanin. In recent years the origin of pigments in vertebrates has been much discussed. One school holds that the epidermis is capable of elaborating its own pigment :<sup>1</sup> another maintains that the pigment is carried into the integument by wandering leucocytes ;<sup>2</sup> still another holds that melanin is derived from the haemoglobin of the blood.<sup>3</sup> For a very complete account of the historical development and the present status of the biological theories regarding the origin of melanin, the reader is referred to Dawson's (1920) paper on the integument of *Necturus*. But, in addition to the purely biological discussion of the question, there are certain chemical investigations on melanin which demand attention.

The chemical analyses which have been made upon melanin tend on the whole to support the view that the pigment is

<sup>1</sup> Hooker (1914) and Eycleshymer (1906) are the more important advocates of this view.

<sup>2</sup> See the papers of Reinke (1906), Nègre (1906), and Borrel (1913).

<sup>3</sup> Rabl (1894) maintained that the leucocytes phagocytized red blood-cells and converted their haemoglobin into melanin. This view is also supported by certain chemical investigations.

a derivative of haemoglobin. In the first place the same elements are present in melanin and in haemoglobin (Hammarsten and Hedin, 1915, p. 84). Aside from nitrogen and sulphur the most noticeable element present is iron. The earlier observers (Scherer, 1841; Berdez und Nencki, 1886) failed to detect iron, but, as Halliburton (1898, vol. i, p. 121) points out, their failure was due to the fact that they extracted the pigment with hydrochloric acid and thus removed the iron. Mörner (1887), and Brandl und Pfeiffer (1890) found that melanin contained a large amount of iron, and believe as a result that melanin is a derivative of the blood-pigments. Schmiedeberg (1897) obtained similar results for the sarcomelanin from a sarcomatous liver, finding that it contained 2.7 per cent. iron. The more recent work on the subject likewise corroborates the observation that iron is present in melanin (Gortner, 1912*a*; von Fürth und Jerusalem, 1907; Piettre, 1911*a*).

There is, therefore, both biological and chemical evidence in favour of the view that melanin is derived from the blood-pigments. Moreover, as the present paper has attempted to show, the great majority of invertebrate pigments are not only derived from the pigments of blood-systems but the invertebrate blood-pigments are themselves derived from food. Unless a profound change has occurred in the physiological processes of the vertebrates as compared with those of the invertebrates—which is not probable—it appears reasonable to the writer to admit that some at least of the vertebrate pigments likewise owe their origin to the pigments of the blood.

**Urochrome.**—The recent feeding experiments of Roaf (1921) have given strong evidence that the output of urochrome from the urine of guinea-pigs and of man is roughly proportional to the amount of chlorophyll taken in as food, and Roaf suggests, in view of the chemical similarity between the two pigments (the pyrrol reaction), that urochrome is derived from chlorophyll. It is evident that this observation throws quite a new light upon the debated question of urinary pigments, and it gives an added instance of the dependence of animals

upon the pigments of plants. The further relation of chlorophyll to the bile and urinary pigments is a subject which will well repay further investigation.

In conclusion it may be said with reasonable certainty that many animals and probably man do normally use the four pyrrol groups of the chlorophyll molecule to synthesize haemoglobin and allied pigments; however, though most evidence points in this direction, no one has actually demonstrated that the animal body is itself incapable of synthesizing haemoglobin in the absence of chlorophyll.

## 8. CONCLUSIONS.

### Part I.

What deductions may be made concerning the animals which have thus far been considered? In the first place it must be recalled that in no case has the writer dealt with forms which have a true blood-vascular system.

The most important function of the blood-system in the higher animals is that of carrying nutriment to the tissues. In the lower invertebrates this function is accomplished either by the direct contact of the tissue with the surrounding seawater, or by circulatory fluids—less highly specialized than blood—which move within the body. These fluids, therefore, are the ones which represent the functional antecedents of the blood-vascular system; and it is to these that one should look in seeking the origin of many of the body-pigments. From the foregoing pages the following conclusions seem reasonable:

1. The pigmented protozoans owe their colour, probably in every case, to an algal pigment which has resulted from an outside infection.

2. Though the evidence in the Porifera is not conclusive,<sup>1</sup>

<sup>1</sup> The evidence recently adduced by Van Trigt (1918) removes any reasonable doubt concerning the chlorophyllous nature of the pigments of a large number of sponges.

it is probable that their pigment is chlorophyll or a substance closely allied (chlorophyll has been demonstrated in more than twenty species of sponge), and there is evidence which indicates that it, too, is obtained from external sources.

3. The constituents from which actinians manufacture their pigment are carried to the tissues by the gastrovascular fluid ; it is likely that the constituents themselves are derived from the chlorophyllous substances which enter as food ; thus they are in a highly-organized state when they reach the tissues, making the synthesis of the pigment less difficult. Certain of the actinian pigments—aside from being derivatives of chlorophyll—are closely related to haemoglobin (actiniohaematin).

4. In certain flatworms which are not coloured by algal symbionts, the pigment is derived from food and is carried to the tissues by the gastrovascular system.

5. The pigment of the red cells of *Tripneustes esculentus*, so numerous in the perivisceral fluid, is identical with the pigment of the epidermis, and since it has been shown by Geddes and others that the pigmented cells arise while in circulation from yellow cells, direct evidence is thereby afforded that the body pigment arises in the nutritive fluid.

6. The red pigment, echinochrome, though probably not respiratory (McClendon), nevertheless bears a close chemical relationship to haemoglobin (Griffiths).

7. Since there is every probability (in *T. esculentus*) that the yellow cells from which the reds arise are chlorophylloid corpuscles, it seems clear that chlorophyll is capable not only of giving rise to an animal pigment but to a pigment which is closely akin to haemoglobin (echinochrome breaks down into haemochromogen, a reduction product of haemoglobin). Bürgi's feeding experiments show that chlorophyll facilitates the formation of haemoglobin in anaemic rabbits.

8. The theory that haemoglobin is derived from chlorophyll is further strengthened by the fact that in many echinoderms there is present, simultaneously with haemoglobin and chlorophyll, a substance, haematoporphyrin, which is an intermediate product chemically between chlorophyll and haemoglobin.

9. In many other echinoderms Asteroidea, Ophiuroidea, Echinoidea, and Holothuroidea, there are found pigmented body-fluid cells which likewise give rise (in part at least) to the external pigmentation by becoming deposited in the epidermis.

## Part II.

In the preliminary observations of the present work, it was found that in nearly every invertebrate investigated there occurs in the blood a chromogen—either completely formed, or in the process of formation—which is similar to, and not infrequently identical with, the pigment of the epidermis. Though this in itself is an interesting fact, it is at once apparent that behind the phenomenon there lies something of much greater significance: Why should the body-pigments occur in the blood-system, and whence do they come? The pigments of the invertebrates, so far as they have been investigated, appear to be derived very largely from food, being absorbed into the blood-stream and carried by that tissue to the epidermal regions, where they are deposited.

The more specific conclusions are as follows:

1. In the absence of a blood-system, as in the Echinoderms and lower forms, the nutritive fluids supply the epidermis with its pigments (Part I).

2. The coloration of several nemertean worms is due to the presence of haemoglobin in the epidermis and in the blood.

3. In certain nudibranchs (*Chromodoris zebra*) and cephalopods, pigments are found in the blood-stream which are identical with the epidermal pigments. There is strong evidence that the pigments of the blood-stream of the Mollusca owe their origin to the chlorophyllous substances taken in as food.

4. The lipochromes of annelids are derived from food substances, being absorbed into the blood-stream and transported to the epidermis. Annelid haemoglobin is found both in the blood and in the epidermis.

5. The enterochlorophyll found in the liver of many crustaceans and molluscs is of vegetable origin. There is evidence

that it is the base from which the animal synthesizes many other of its pigments, including haematin.

6. The red lipochrome of the blood and the chromatophores of crustaceans are derived from carotin, a pigment associated with chlorophyll (found also in the liver). Carotin and the red lipochrome of crustaceans are chemically identical (Verne).

7. Many pigments of insects are modified chlorophylls derived directly from the chlorophyll of the food (Poulton).

8. The pigments of the tunicates are found first in the blood-system. The pigmented cells arise, while in the circulation, from unpigmented corpuscles, and certain of the pigment cells which arise in this way are subsequently deposited in the test.

9. Strong evidence exists that the respiratory pigment haemoglobin is derived both phylogenetically and physiologically from chlorophyll.

I cannot close this paper without mentioning my great indebtedness for advice and inspiration to Professor Benjamin Moore whose recent untimely death will be most keenly felt in America as well as in England. He more than any one has helped to clarify the perplexing question of the relation which chlorophyll bears to the protoplasmic system of plants and animals.

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